a fully-automated data acquisition system to collect a very large number of photon trajectories at high illumination intensities, and carried out a collective photon-by-photon analysis of the transitions between the folded and unfolded states using a maximum likelihood method (Chung et al., JPC A 2011). We determined a transition-path time of ~2 microseconds for a WW domain that folds in ~100 microseconds and an upper bound of ~15 microseconds for protein GB1 that folds in ~2 seconds. The transition-path times for the two proteins differ by less than 10-fold while the folding rates differ by a factor of 20,000. This result shows that a slow-folding protein can fold almost as fast as a fast-folding protein when folding actually occurs!

1099-Plat
Influence of Calcium Binding on the Folding Properties of Single Calmodulin Molecules Observed with Optical Tweezers
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Calcium sensing and the control of signaling pathways play important roles in the control of cellular processes, ranging from immune response to apoptosis. The calcium-dependent signal transducer calmodulin is one of the key players involved. Relatively unstable at low calcium concentrations, calmodulin is greatly stabilized upon calcium binding, undergoes a conformational change and is able to bind other proteins for a specific response.
We use a dual beam high resolution optical tweezers setup to investigate the folding/unfolding properties of single calmodulin molecules. Using force both as a denaturant and a reporter for molecular extension we can tune the equilibrium between folded, intermediate, and unfolded states. Already in relatively small multi-domain proteins such as calmodulin, complex networks with on and off-pathway states can be found. We investigate the equilibrium fluctuations of calmodulin over several minutes and directly observe of the effects of calcium binding on the folding process.

1100-Plat
Cavities in the Hydrophobic Core Govern Pressure Unfolding of Proteins
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Since Bridgman's seminal experiments on high-pressure denaturation of albumen in 1914[1], the origin of pressure unfolding of proteins has remained unresolved. We report here a systematic study of the contribution of cavities to the volume difference between unfolded and folded states (ΔV_u), using 10 single point variants of staphylococcus nuclease (SNase). Each mutation is localised in a strategic position and was designed to change a large buried hydrophobic side chain into alanine, thus opening tunable cavities in the SNase structure. For every variant, a crystal structure confirmed the presence of the designed cavity with no detectable presence of water molecules. High-pressure fluorescence experiments show significantly larger ΔV_u values for the cavity mutants in comparison to the reference protein. This demonstrates that solvent-excluded cavities make a major contribution to ΔV_u. Thus, pressure effects have their origin in a property of the folded states of proteins, unlike temperature and chemical denaturants, whose effects are governed by exposed surface area in the unfolded state. High-pressure NMR experiments on 4 cavity mutants, recording HSQCs peak intensities up to 2500 bar, allowed precise estimations of the apparent ΔV_u monitored by more than two-thirds of the residues. An innovative combination of the site-specific NMR data and Go-model simulations revealed significant departures from the apparent two-state folding process for the SNase reference protein and the cavity mutants. This study opens up highly promising perspectives on the use of high pressure for characterization of folding landscapes inaccessible by other methods.
1. Bridgman, P. W. *J. Biol. Chem.* 1914.19, 511–512.

1101-Plat
The Fast and the Slow: Folding and Trapping of λ6-85
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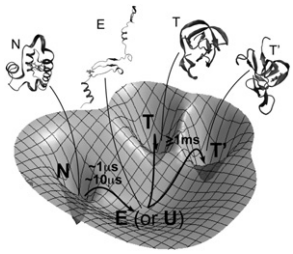
Molecular dynamics simulations that combine many μs trajectories have recently predicted that a very fast-folding protein like lambda repressor fragment $\lambda_{6\text{-}85}$ D14A could have a millisecond kinetic phase. We investigated this possibility by detecting temperature jump relaxation to 5 ms. While $\lambda_{6\text{-}85}$ D14A shows no significant slow phase, two even more stable mutants do. A slow phase of $\lambda_{6\text{-}85}$ D14A does appear in mild denaturant. The experimental data (and we believe also the computational modeling) is consistent with the following hypothesis: $\lambda_{6\text{-}85}$ takes only microseconds to reach its native state from an extensively unfolded state, while the latter takes milliseconds to reach compact traps containing beta sheet structure. $\lambda_{6\text{-}85}$ is not only thermodynamically, but also kinetically protected from reaching intramolecular analogs of beta sheet aggregates while folding.



1102-Plat
Membrane Protein Stabilities and M-Values
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Understanding and prediction of membrane protein structures requires knowing the physical forces stabilizing them. However, such measurements are rare for membrane proteins. The few measurements that have been made were carried out under very different experimental conditions using different lipid bilayer compositions and geometries, which makes derivation of sequence-structure-energy relationships difficult. We have overcome many technical obstacles to measuring folding free energies of membrane proteins and will present novel measurements of the free energy of unfolding and the m value for several membrane proteins from E. coli. These stability measurements were accomplished in the same lipid bilayer system, and our results indicate that the trends in the stability of those proteins can be explained by the degree of hydrophobicity of their lipid-facing residues in their transmembrane regions. We also found that the sensitivity of these membrane proteins to chemical denaturation (as judged by their m values) was consistent with the sensitivity of water-soluble proteins having comparable differences in the solvent-exposure between their folded and unfolded states.

1103-Plat
Quantifying the Dimerization Energy of a CLC Transporter in Lipid Bilayers
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Recently, a structurally stable and functional monomeric form of the normally homodimeric Cl^-/H^+ antiporter CLC-ec1 was designed by introducing two tryptophan mutations at the dimer interface, I201W and I422W. Several single tryptophan mutant constructs show intermediate stability between monomer and dimer state, as observed by size exclusion chromatography. In addition, the monomer and dimer populations can be shifted by adding lipids to the purified protein in detergent micelles, indicating that the system is undergoing reversible dimerization. We are now developing CLC-ec1 into a model for measuring dimerization energetics in a lipid bilayer. To measure the free energy, as well as enthalpic and entropic contributions, we must determine the fraction of monomer and dimer in the total protein population at different temperatures. To do this, we introduce cysteine residues into the extracellular loop adjacent to the dimerization interface, and fluorescently label the protein with tetramethylrhodamine- (TMR) or fluorescein-maleimide. The fluorescent protein is then reconstituted into liposomes at known concentration, and the fraction of dimer is measured in one of two ways. In the first method, protein is labeled with TMR at a position that allows the rhodamine molecules to undergo self-quenching by forming non-fluorescent pairs when in the dimer state. Addition of 0.5% SDS dissociates the dimer, as confirmed by glutaraldehyde cross-linking, leading to an increase in fluorescence at the rhodamine peak emission wavelength, and a measurement of the dimer population. In the second method, protein is co-labeled with fluorescein and TMR, and the Förster resonance energy transfer signal is measured from the dimer complexes. These studies introduce CLC-ec1 reversible dimerization as a simplified model for the thermodynamics of membrane protein folding and TM helix recognition in the membrane environment.

1104-Plat
Cysteine Shotgun-Mass Spectrometry (CS-MS) Reveals Dynamic Sequences of Protein Structure Changes within Mutant and Stressed Cells
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Questions of if and when protein structures change within cells pervade biology and include questions of how the cytoskeleton sustains stresses exerted on or by cells _particularly in mutant versus normal cells. Cysteine shotgun labeling with fluorophores is analyzed here with mass spectrometry of the spectrin-actin membrane skeleton in sheared red blood cell ghosts from normal and diseased mice. Sheared samples are compared to static samples at 37 °C in terms of cell membrane intensity in fluorescence microscopy, separated protein fluorescence, and tryptic peptide modification in liquid chromatography-tandem mass spectrometry (LC-MS/MS). Spectrin labeling proves to be the most

sensitive to shear, whereas binding partners ankyrin and actin exhibit shear thresholds in labeling and both the ankyrin-binding membrane protein band 3 and the spectrin-actin stabilizer 4.1R show minimal differential labeling. Cells from 4.1R-null mice differ significantly from normal in the sheardependent labeling of spectrin, ankyrin, and band 3: Decreased labeling of spectrin reveals less stress on the mutant network as spectrin dissociates from actin. Mapping the stress-dependent labeling kinetics of α- and β-spectrin by LC-MS/MS identifies Cys in these antiparallel chains that are either force-enhanced or forceindependent in labeling, with structural analyses indicating the force-enhanced sites are sequestered either in spectrin's triplehelical domains or in interactions with actin or ankyrin. Shearsensitive sites identified comprehensively here in both spectrin and ankyrin appear consistent with stress relief through forced unfolding followed by cytoskeletal disruption.

1105-Plat
Expanding Anfinsen's Principle: Controlling Protein Structure by Altering Local Translation Rate
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Anfinsen's seminal experiments on the refolding of ribonuclease *in vitro* showed that the final native structure of a protein is determined by its primary amino acid sequence. *In vivo,* a nascent polypeptide chain can start to fold co-translationally, and the conformations adopted during chain synthesis can be distinct from the conformations adopted by free, full length chains refolded after dilution from denaturant. Moreover, altering protein translation rate has been shown to further affect co-translational protein folding mechanisms. Translation rate can be altered without changing the encoded amino acid sequence by altering synonymous codon usage. But common codons are typically translated faster than rare codons, and clusters of rare codons can cause significant pauses in translation. Here, we designed and tested a system to investigate the effects of a cluster of synonymous rare codons on a protein folding mechanism and its final folded structure. We designed a protein that consists of three half-domains, connected by flexible linkers. The N- and C-terminal half-domains compete to interact with the central half-domain. This protein therefore has the potential to form one of two mutually exclusive native structures. We altered synonymous codons at the 5' end of the sequence encoding the C-terminal half-domain to adjust the rate at which the C-terminal half-domain appears outside the ribosome exit tunnel. The presence of rare codons at this location causes the N-terminal half-domain to pair with the central domain more often than the C-terminal half-domain. This effect is tunable, based on the rareness of the codons and the corresponding duration of the translation pause. These results suggest that Anfinsen's principle might need to be expanded, to include possible effects of translation rate on protein structure formation.
Either

Platform: Cell & Bacterial Mechanics & Motility I

1106-Plat
Mitotic Spindle in HeLa Cell Rotates According to the Local Temperature Gradient
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The temperature gradient is present at the surface of living organisms, and also inside of the bodies. Each cell has to adapt to the change in the temperature over short and long timescale to precisely perform cellular processes. Ishizaka has reported that the position of mitotic spindle in grasshopper spermatocyte shifted under temperature gradient, resulting in unequal cell division (Ishizaka, S. Dev. Growth Differ. 11, 179–85, 1969). Here we created the temperature gradient within single HeLa cells in mitosis, and show that the mitotic spindle is rearranged so that the pole-to-pole axis is to be perpendicular to the gradient. Our results suggest that one of the key components of this response is the temperature-dependent polymerization dynamics of microtubule cytoskeleton, a scaffold of the spindle. Then, we further confirmed that the elongation rate was increased according to the temperature gradient in the spindle. Due to this anisotropy in the astral microtubules, these microtubules could produce pushing and pulling forces such that the pole closer to the heat source can move down the slope of the temperature until the temperature at this pole is equal to that at another.

1107-Plat
The Role of the Contractile Ring during Cytokinesis
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Cytokinesis in animals and fungi involves constriction of an actomyosin contractile ring, but the constriction mechanisms and the role of the ring are not established. The constriction rate could be determined only by the properties and internal dynamics of the ring itself (a ''dynamically autonomous'' mechanism) as suggested by recent experiments on C. elegans embryos. Alternatively, the ring constriction rate could be set by coupled processes that occur simultaneously with constriction (a ''dynamically coupled'' mechanism). In fission yeast constriction occurs simultaneously with septation, the poorly understood process of cell wall growth in the wake of the constricting ring. To isolate the ring constriction mechanism, we combined mathematical modeling with experiments on fission yeast protoplasts which lack cell wall and adopt a rounded shape. Protoplasts assembled functional contractile rings that constricted without septation by sliding along the plasma membrane without dividing the cell. Because we could manipulate the shapes of protoplast cells, we could test the influence of cell shape on ring constriction dynamics and distinguish between dynamically autonomous and dynamically coupled constriction dynamics. In compressed protoplasts that had a partially flattened shape, contractile rings adopted characteristic bent shapes during constriction that were in remarkably close quantitative and parameter-free agreement with a mathematical model that assumed the ring produces tension but its constriction rate is set by the sliding of ring anchors in the membrane. Thus, ring constriction in fission yeast protoplasts is dynamically coupled: the ring does not set its own constriction rate. Dynamically autonomous models could not reproduce our experimental observations. Our results suggest that in normal yeast cells the constriction rate is determined by the septum growth rate or other coupled process and the role of the ring could be to exert tension on the septum to regulate its growth.

1108-Plat
Material Properties of Actin Networks from Motile Fish Keratocytes under Large Deformations
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In actin-based crawling motility, cells continuously build, reorganize, and disassemble an actin network in a process driven jointly by biochemical reactions and mechanical work. A thorough understanding of how forces produced by actin and myosin contribute to whole-cell movement will thus require detailed knowledge of the material properties of the cytoskeletal network at the relevant spatial and temporal scales (micrometer-scale deformations over tens of seconds to minutes). Measurements of mechanical properties have largely been limited to microscopic strains, whole-cell bulk measurements, or reconstituted gels that do not fully capture the cellular cytoskeletal organization.
We have therefore sought to characterize the deformation of actin networks driven from motile cells under large (up to several hundred percent) applied strains. Using detergent-extracted cytoskeletons from fish epithelial keratocytes, we have applied arbitrary strains to the actin network between the cell body at the rear of the cell and the native adhesions in the lamellipodial (front) region of the cell, using a glass needle. Deformations through the cell body propagated through a significant portion of the lamellipodium, in some cases reaching the leading edge, indicating good mechanical linkage between the cell body and the lamellipodium. The lamellipodial actin network is surprisingly flexible and exhibits strain hardening. At rates of deformation comparable to the speeds of live, crawling cells, the network exhibited significantly elastic behavior, suggesting that elastic forces might contribute to the myosin-driven reorganization of the actin network in the rear of the cell. These results will help refine current physical models for crawling cell motility.

1109-Plat
Linking Molecular Transport, Traction Forces and Signaling in Migrating Cells
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Cell migration is a vital process in which cells undergo directed movement to particular locations. However, the molecular mechanisms that coordinate this process are still poorly understood. Here, two techniques are used in tandem